The immunological consequences of photodynamic treatment of cancer, a literature review

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Abstract

In this review we discuss the effect of photodynamic treatment (PDT) of solid tumors on the immune response. The effect on both the innate and adapted immune response is discussed. We have summarized the evidence that PDT causes or enhances an anti-tumor response. PDT is a local treatment in which the treated tumor remains in situ while the immune system is only locally affected and still functional in contrast with e.g. after systemic chemotherapy. We conclude that PDT of cancer is a way of in situ vaccination to induce a systemic anti-tumor response. In general, immune cells are found in the tumor stroma, separated from tumor cells by extracellular matrix and basal membrane-like structures. We hypothesize that PDT destroys the structure of a tumor, thereby enabling direct interaction between immune cells and tumor cells resulting in the systemic anti-tumor immune response.

Abbreviations: ALA = 5-aminolaevulinic acid; APC = antigen presenting cells; CHS = contact hypersensitivity response; DBPMAF = vitamin D₃-binding protein derived macrophage-activating factor; G-CSF = granulocyte colony stimulating factor; HpD = haematoporphyrin derivative; PDT = photodynamic therapy; PIT = photoimmunotherapy

Introduction

Tumor cells and the nourishing microvasculature are the targets of photodynamic therapy (PDT), resulting in primary tumor cell death via a direct and indirect pathway. Direct tumor cell death occurs when during light irradiation, energy is transferred from the excited photosensitizer onto oxygen molecules, generating the formation of singlet oxygen (type II photochemical reaction). This highly reactive molecule causes direct photodamage of proteins, lipids and other molecules at the sites where the photosensitizer accumulates, leading to PDT-mediated direct tumor cell killing either by apoptosis or necrosis. The mode of cell death upon PDT depends on several factors. The intracellular localization of the photosensitizer is of great importance, since damage to mitochondria generally leads to apoptosis, whereas plasma membrane damage can delay or even inhibit apoptosis and instead induces necrosis (Kessel et al., 1997; Luo & Kessel, 1997; Kessel & Luo, 1998; Chen et al., 2000; Fabris et al., 2001). Haematoporphyrin derivative (HpD) and its purified form Photofrin, one of the most widely used...
photosensitizers, localize in mitochondria due to both their hydrophobicity and the affinity for a plasma binding site on the mitochondrial membrane that also binds benzodiazepines (Roberts & Burns, 1989; Shulok et al., 1990; Wilson et al., 1997). Another often-used drug in PDT is 5-aminolevulinic acid (ALA). This is not a photosensitizer, but a precursor of the endogenous photosensitizer protoporphyrin IX in the haem synthesis pathway, which is produced in the mitochondria (Liang et al., 1998; Chen et al., 2001). Other more hydrophilic sensitizers such as phthalocyanines and many chlorines enter the cells via endocytosis and hence accumulate mainly in lysosomes. When cells are illuminated, relocalization occurs throughout the cytosol (Wood et al., 1997; Ball et al., 1999; Pogue et al., 2001). Also the dose of photosensitizer and/or light influences the occurrence of either necrosis or apoptosis. For apoptosis the presence of functional enzymes is necessary, therefore if PDT dose is too high, apoptosis cannot occur. This is confirmed by several researchers who showed that low dose in vitro PDT results in apoptosis whereas high dose PDT leads to necrosis (Oleinick & Evans, 1998; Lavie et al., 1999; Di Stefano et al., 2001; Vantieghem et al., 2001). Studies with apoptosis inhibitors and with cell lines that lack functional caspase-3 show that when the apoptotic pathway is blocked, necrosis occurs instead so the overall effect of treatment is still the same (Wyld et al., 2001; Thibaut et al., 2002). However, there may be differences in the occurrence of a systemic immune response, since necrosis more than apoptosis leads to inflammation, which is the first step in induction of a specific immune response.

For complete eradication and long-term control of tumors, indirect tumor cell killing seems as important as direct cellular damage. Phototoxic lesions in the endothelium of tumor blood vessels lead to severe and persistent post-PDT tumor hypoxia/anoxia (Star et al., 1986). These vascular effects of PDT are caused by reversible contraction of endothelial cells resulting in the exposure of the basement membrane, macromolecular vessel leakage, leucocyte adhesion and thrombus formation (Nelson et al., 1988; Fingar et al., 1992). All are apparently linked to platelet activation and the release of thromboxane (Fingar et al., 1990, 1993). PDT may also inhibit the production or release of nitric oxide by the endothelium, leading to more vessel constriction (Gilissen et al., 1993). This chain of events leads to complete vascular occlusion and the ischemic

![Schematic representation of direct and indirect courses of tumor destruction upon PDT, as explained in detail in the text.](image-url)
death of remaining tumor cells. A schematic representation of how tumors are destroyed by PDT is shown in figure 1.

**Induction of an Anti-tumor Immune Response by PDT**

After this primary tumor cell death, there may be a secondary cause of tumor cell death, mediated by a systemic anti-tumor immune response induced after PDT. Animal models have shown that this may be an additional effect of PDT of cancer.

PDT of EMT6 mammary sarcoma in SCID and normal mice showed a significantly lower therapeutic effect in SCID mice, suggesting that the difference in tumor cures originated in the lack of an immune response (Korbelik et al., 1996). This effect could be restored by adoptive transfer of T-lymphocytes of normal (BALB/c) mice into the SCID mice. Hendrzak-Henion and colleagues (1999) found similar results in a study with T cell deficient mice. In a later study, Korbelik and Celic (1999) selectively depleted EMT6 sarcoma-bearing mice of specific myeloid (neutrophils, macrophages) and lymphoid (T cells) populations. Although immunodepletion of T cells did not affect the initial tumor ablation by PDT, the tumor cure rate was significantly reduced, showing the importance of the immunological contribution to the effect of PDT.

The hypothesis is also supported by findings of regression of untreated metastases at a distant site from the treated primary tumors (Chen et al., 1999). Accordingly, tumor rechallenge in rats effectively treated with PDT did not lead to tumor growth suggesting that an immunological memory had been generated by the initial PDT treatment. This tumor immunity appeared to be transferable (Korbelik et al., 1996).

These studies confirm the existence of a systemic anti-tumor response after PDT, in which T cells play a crucial role.

**How Does PDT Affect the Immune System?**

**Direct effect of PDT on immune cells**

The direct effect of PDT on immune cells may be harmful. Lymphocytes, especially activated T cells, accumulate photosensitizer and can therefore be killed by subsequent illumination, leading to a local suppression of the immune system (Hryhenko et al., 1998; Hunt et al., 1999; Casas et al., 2002).

One of the parameters to measure this effect of PDT on immune cells is a contact hypersensitivity response (CHS), a classical example of a cell-mediated delayed-type immunologic reaction with epidermal Langerhans cells, macrophages, and T lymphocytes participating. In the 1980's Elmets and Bowen (1986) already described the suppression of this response upon PDT treatment of the skin. A study by Gollnick et al. (1997) indicated that an increase in IL-10 might be responsible for this immunosuppressive effect of PDT, but a later study by the same author showed that suppression of CHS upon cutaneous PDT did not occur via an IL-10 dependent mechanism (Gollnick et al., 2001a). Transcutaneous whole body PDT however was shown to be IL-10 dependent by Simkin et al. (2000). An adoptive transfer study in mice by Jolles et al. (1988) showed that the reduction of the CHS also extended to areas other than the illuminated site. This phenomenon was associated with the generation of suppressor cells and an acute-phase response, characterized by leucocytosis and elevated serum amyloid P levels. In a study by Qin et al. (1993) allograft survival was prolonged after pretreatment of the peritoneum with HpD-PDT. Nearly complete depletion of peritoneal lymphocytes was observed in mice after PDT treatment of the exposed musculoperitoneal layer, but also a loss of responsiveness of splenic lymphocytes to mitogens was found. In *vitro* PDT of macrophages had a similar harmful effect, leading to decreased viability (Steubing et al., 1991; Reiter et al., 1999).

In another study on allograft survival and the immunosuppressive effects of PDT, Obochi and co-workers (1997) propose that low-dose PDT of donor skin tissue induces a series of down regulatory events in the graft, resulting in decreased expression of MHC and costimulatory molecules on the surface of donor Langerhans cells. King et al. (1999) found a similar reduction in expression of MHC antigens and costimulatory molecules such as CD80 and CD86 on mouse dendritic cells after treatment with PDT. As a result of this decreased expression of surface MHC class II molecules, Langerhans cells largely lose the ability to stimulate the efficient proliferation of allogenic T-cells. This impairment of antigen presenting capacity was also demonstrated by Hryhorenko and co-workers (1998) who showed in an *in vitro* study with human peripheral blood monocytes in a mixed leucocyte reaction that the ability of antigen presenting cells (APC) to properly present antigens to T cells significantly decreases.
after treatment of APC with PDT. Similar findings were published by Barret and Gruner, with protoporphyrin and HpD-based PDT respectively (Gruner et al., 1986; Barrett et al., 1994). These studies thus show the antigen presenting capacity of monocytes and dendritic cells to decrease after PDT, resulting in decreased contact hypersensitivity response and increased allograft survival.

Systemic effects on the immune system upon local PDT tumor treatment

In general, immune cells are found in the tumor stroma, separated from tumor cells by extracellular matrix and basal membrane-like structures i.e. there is no or minimal direct interaction (Hagenaars et al., 2000; Kuppen et al., 2001). It is conceivable that PDT destroys the structure of a tumor, thereby enabling direct interaction between immune cells and tumor cells. After the initial PDT-induced damage to tumor cells and immune cells, indeed a strong inflammatory reaction occurs locally, which leads to influx and activation of undamaged immune cells from elsewhere.

Photodamage of membrane lipids results in the activation of membranous phospholipases, leading to a massive release of lipid fragments and metabolites of arachidonic acid (Henderson & Donovan, 1989; Agarwal et al., 1993). Both are potent inflammatory mediators. Furthermore, changes in the blood vessel walls, as described above (e.g. exposure of the basement membrane), will attract neutrophils and platelets (de Vree et al., 1996a). These cells will also release more inflammatory mediators, which chemotactic capacities enable a massive recruitment of immune cells to the damaged site. PDT is associated with elevated expression and/or production of several cytokines: IL-1β, IL-2, IL-6, IL-10, TNF-α and granulocyte colony-simulating factor (G-CSF) (Herman et al., 1996; Gollnick et al., 1997; Ziolkowski et al., 1997; Chen et al., 2000; Blank et al., 2001; Gollnick et al., 2001b; Usuda et al., 2001). These cytokines are important messenger proteins, regulating the inflammatory and immunological responses towards bacteria, viruses and tumors. Release of these chemotactic factors from affected tissue results in the attraction and accumulation of non-lymphoid inflammation cells. Within minutes after PDT treatment a large number of neutrophils invade the area (Kros et al., 1995; Gollnick et al., 1997). Most likely, these phagocytes are present at the inflammatory site to remove cell debris caused by PDT. Neutrophils appear to be at least partially dependent on an IL-1 induced release of granulocyte colony stimulating factor (G-CSF) (de Vree et al., 1997). De Vree et al. (1996b) found that PDT of rhabdomyosarcoma-bearing rats resulted in neutrophilia and an increase in IL-1 serum levels. Administration of anti-G-CSF antibody led to a decrease in PDT efficacy. Although elevated neutrophil levels were also found in control rats, which received surgery only, a delay in tumor growth was not detected here. It was concluded that PDT with the help of IL-1 and G-CSF had a stimulatory effect on neutrophil activity, turning them into direct tumor cell killers. The importance of the role of neutrophils in the anti-tumor effect of PDT was also shown by depletion of neutrophils in mice directly after PDT treatment of subcutaneous EMT6 mammary sarcoma, which resulted in 70% reduction of the curative effect, as measured by the percentage of tumor-free mice after 90 days. Depletion of neutrophils did not affect the initial tumor ablation, induced within the 24 hours after PDT (Korbelic & Cecic, 1999).

Neutrophil invasion is followed by the arrival of mast cells, lymphocytes, monocytes and macrophages (Kros et al., 1995). Although direct in vitro PDT of macrophages results in decreased viability of these cells (Steubing et al., 1991; Reiter et al., 1999), several researchers described an increase in phagocytic capacities when low drug and light doses are administered in both in vitro and in vivo studies (Yamamoto et al., 1994; Coutier et al., 1999). A point of discussion is whether PDT activates macrophages directly, or indirectly via increased susceptibility of tumor cells to phagocytosis. Reiter et al. (1997) found support for the indirect activation of macrophages since in their studies, Hpd-PDT treated macrophages did not exhibit increased cytotoxicity against YAC-1 murine T-lymphoma cells whereas photodynamic treatment of tumor cells did result in activation of tumoricidal effector functions of macrophages. A later study showed that when PDT-treated carcinoma cells were added in vitro to macrophages, these macrophages became more cytotoxic to other carcinoma cells, which fortified the hypothesis that macrophages are stimulated by damaged tumor cells rather than by PDT (Reiter et al., 1999). Yamamoto et al. (1991) however found that in vitro PDT of macrophages only led to higher ingestion activity when B- and T-lymphocytes were present together with the macrophages during PDT, suggesting a mechanism whereby damage to cell membranes of lymphocytes leads to activation of macrophages. In two other studies, macrophages were harvested from PDT-treated tumors and from the PDT-treated peritoneum. These macrophages exhibit increased cytolytic capacities, but since both lymphocytes and tumor cells were
present in this in vivo situation, these studies seemingly can not sufficiently refute the aforementioned results (Qin et al., 1993; Kros et al., 1995). As macrophage inactivation with silica particles injected immediately post-PDT intra-peritoneally in mice with EMT6 sarcoma resulted in 40% less tumor-free mice after 90 days, it can be assumed that their role in PDT efficacy is considerable (Korbelik & Cecić, 1999).

After phagocytosis and processing of tumor cell debris, macrophages may function as APC (Ziegler & Utanue, 1981). In contrast to e.g. radiotherapy, PDT induces a rapid release of tumor cell debris that may enhance the uptake and presentation of tumor antigens by tumor-associated APC and thus lead to lymphocyte involvement, as shown by infiltration of vulval intraepithelial neoplasia with cytotoxic T lymphocytes after treatment with ALA-based PDT (Abdel-Hady et al., 2001). The ensuing systemic immune response may result in destruction of locally remaining tumor cells as well as prevent the occurrence of distant metastases. This is adequately shown by animal studies in which treatment of the primary tumor with PDT resulted in a decrease in distant metastases when compared to animals of which the tumors were resected or not treated at all (Gomer et al., 1987; van Hillegersberg et al., 1995; Schreiber et al., 2002). Chen et al. (1999) showed this anti tumor effect not only on newly formed metastases but also on established metastases in a rat model, although it must be noted that in this study the immunoadjuvant glycated chitosan was linked to the photosensitizer. Rats were implanted in this study with metastatic mammary tumor cells in inguinal fat pads, resulting in primary tumors after 5 days that metastasised to remote inguinal and axillary regions after 15–20 days. After successful PDT treatment of the primary tumors, rats were rechallenged 120 days after initial inoculation with 10^6 tumor cells. PDT treatment resulted in disappearance of the metastases of the primary tumor as well as in total resistance to rechallenge. Accordingly, Korbelik et al. (1996) found that tumor rechallenge in rats effectively treated with PDT did not lead to tumor growth suggesting that an immunological memory had been generated by the initial PDT treatment. This tumor immunity appeared to be transferable. These studies strongly suggest the induction of a systemic anti-tumor immune response after PDT. Since local PDT treatment enhances or causes this systemic immune response, it could be regarded as a way of in situ vaccination. An important factor in this may be that PDT causes the destruction of extracellular matrix that surrounds established tumor nodules and thus enables interaction between immune cells and tumor cells (Hagenaars et al., 2000).

### Photoimmunotherapy

The developing field of photoimmunotherapy (PIT), a combination of PDT and immunotherapy, deserves special interest. This combination aims at potentiating the cytotoxic effect of PDT by improvement of the tumor specific localisation of the photosensitizer and/or by boosting specific parts of the immune system.

Selective treatment of tumors may be achieved by binding of the photosensitizer to a tumor-specific antibody. Del Governatore et al. (2000) performed an in vivo study with a chlorin-e6/Mab 17.1A conjugate, directed against the antigen EpCAM that is associated with carcinomas of epithelial origin. Tumor reduction and median survival were significantly higher in the PIT treated rats when compared to immunotherapy or PDT alone.

PIT may also consist of PDT combined with a specific immuno-enhancer such as granulocyte-macrophage colony stimulating factor (GM-CSF) (de Vree et al., 1997). This cytokine controls the maturation and function of granulocytes and macrophages, and also stimulates the proliferation and differentiation of dendritic cells and other antigen-presenting cells. Reports by Golab et al. (2000) and Kros et al. (1996) both demonstrate a substantially improved curative anti-tumor effect of PDT when tumor-localized GM-CSF immuno-adjuvant treatment was administered. The same effects were found when vitamin D3-binding protein-derived macrophage-activating factor (DBPMAF) (Korbelik et al., 1997) and glucan schizophyllan (Kros & Korbelik, 1994) were used in vitro. It is suggested that PDT is highly receptive to immuno-adjuvant therapy with a macrophage-activating factor, thus fortifying the hypothesis that the immune system plays an important role in the long-term tumor eradication after PDT.

Immune potentiation can also be found when applying PDT in vitro to a transfected IL-6 producing Lewis lung carcinoma cell line, which led to increased sensitivity of cells to PDT as measured by MTT assay. A combination of PDT and IL-6 administration may therefore be able to enhance the anti-tumor effect of PDT (Usuda et al., 2001). Korbelik and co-workers (2001) showed higher cure rates for PDT of human cervical squamous cell carcinoma and human colorectal adenocarcinoma in mice when combining PDT with adoptive transfer...
of IL-2 producing NK-cells. Transfected IL-2 producing NK cells were administered peritumoral or intravenously immediately after PDT, resulting in improved cure rates when compared to PDT treatment without NK-cells or with non-IL-2 producing NK cells.

Conclusion

As PDT locally damages the tumor structure, it enables influx of non-affected immune cells into the PDT site, resulting in the development of a systemic anti-tumor immune response. The immune system should therefore be protected from harmful effects of PDT, for example by applying tumor treatment locally only, preferably in combination with immune enhancing treatment, e.g. by using systemically applied cytokines or immune modulators as GM-CSF, G-CSF, DPBMAF and glucan schizophyllan. However, clinical trials are necessary to assess the applicability of these experimental in vivo and in vitro combination therapies. Future research must be focussed on PDT as a way of in situ vaccination in cancer therapy to cure and prevent the formation of distant metastases.

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